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Synthesis and Conformational Analysis of 2′-Fluoro-5-methyl-4′-thioarabinouridine (4′S-FMAU)

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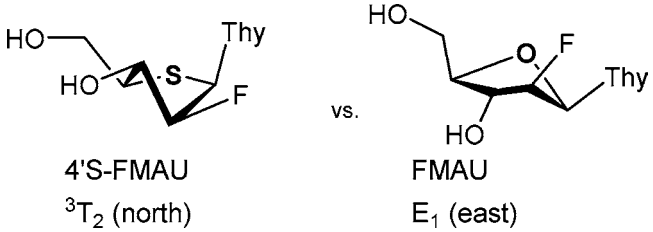

An improved synthesis of 2′-deoxy-2′-fluoro-5-methyl-4′-thioarabinouridine (4′S-FMAU) is described. Participation of the 3′-*O*-benzoyl protecting group in the thiosugar precursor influenced the stereochemistry of the N-glycosylation reaction in nonpolar solvents, permitting a higher β/α ratio than previously observed for similar Lewis acid catalyzed glycosylations. Conformational analysis of the nucleoside using $^3J_{HH}$ and $^3J_{HF}$ NMR coupling constants together with the PSEUROT program showed that it adopted a predominantly northern conformation in contrast to 2′-deoxy-2′-fluoro-5-methylarabinouridine (FMAU), whose PSEUROT conformational analysis is presented here for the first time, which showed a dominantly southeast conformation. The sharp conformational switch attained by replacing the ring heteroatom is attributed to a decrease in relevant steric and stereoelectronic effects.

Introduction

The conformation of oligonucleotides is believed to depend strongly upon the conformation of the nucleotide monomers that make them up.[1] Thus, to understand and design oligonucleotide therapeutics successfully, it is essential to be able to understand and manipulate nucleoside conformations.

As part of our ongoing program to develop new nucleic acid chemistries, we chose to replace the 4′ oxygen of 2′-deoxy-2′-fluoro-5-methylarabinouridine (FMAU) with a sulfur atom. On a chemical level, it was envisaged that this modification would modulate stereoelectronic and steric effects in the 2′-fluoro-arabinofuranose moiety. We were especially interested in investigating the effect of the 4′-thio modification in conjunction with the presence of the 2′-fluorine, which led to favorable biological properties in the case of the 2′F-ANA oligonucleotides.[2] Although 2′-deoxy-2′-fluoro-4′-thioarabinonucleosides have previously been synthesized,[3−6] this is the first time their conformations have been examined.

Results and Discussion

Synthesis. 2,3,5-Tri-*O*-benzyl-1,4-anhydro-4-thio-arabinitol (1) was prepared from L-xylose following a procedure similar to that of Satoh et al.[7] The benzyl protecting groups were removed by Birch reduction using Li/liq NH_3 to give triol **2**.[8]

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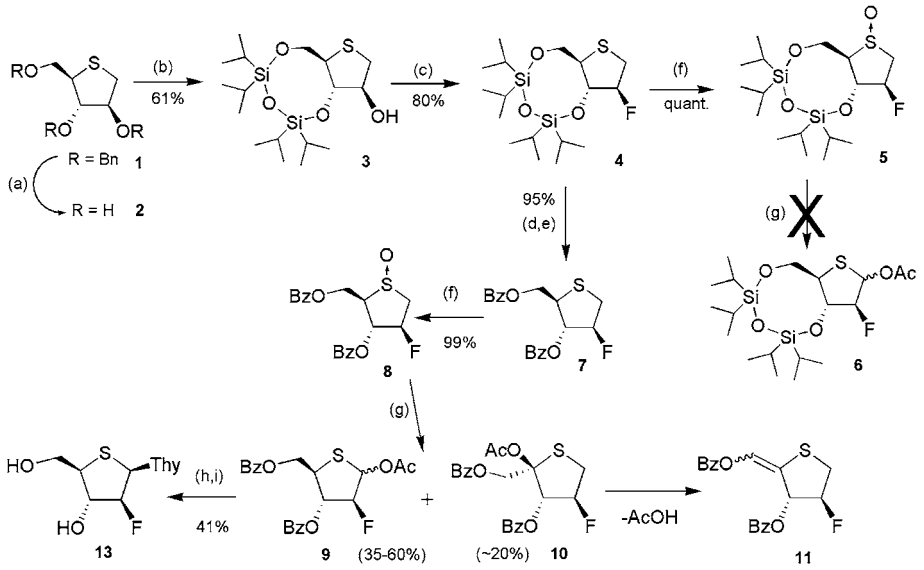
SCHEME 1[a]



[a] Reagents and conditions: (a) Li, liq NH_3, −78°C; (b) $TIPSCl_2$, pyridine, rt, 3 h; (c) DAST, CH_2Cl_2, −15°C, 15 min; (d) Bu_4NF, THF, rt, 30 min; (e) BzCl, pyridine, rt, 6 h; (f) O_3, CH_2Cl_2, −78°C, 30 min; (g) Ac_2O, 110°C, 3 h; (h) bis-silylated thymine, TMSOTf, CCl_4, reflux, 16 h, 47% yield of β product; (i) 2M NH_3 in MeOH, rt, 23 h, 87%.

Treatment of triol **2** with equimolar ratios of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPSCl_2$)[9] in pyridine gave mainly the desired compound **3** that, when treated with DAST, gave within 10 min the desired 2-fluoro derivative **4** in 80% yield. Moreover, the reaction proceeded with retention of configuration, presumably through an episulfonium ion intermediate.[4,10]

To install the pyrimidine base at C-1, we chose to functionalize C-1 as an acetate derivative through the Pummerer reaction, as reported by Naka et al.[9] Thioether **4** was thus subjected to ozonization at −78 °C to give sulfoxide **5** quantitatively. When compound **5** was treated with Ac_2O at 70 °C, several components were observed on TLC, suggesting that the silyl protecting group was being removed. We therefore decided to replace the 3,5-*O*-disiloxane bridge with benzoyl protecting groups. Thus, thioether **4** was treated with Bu_4NF followed by BzCl in pyridine to give compound **7** in excellent yield. Ozonization of thioether **7** at −78 °C afforded sulfoxide **8** that, when treated with Ac_2O at 110 °C, gave mainly the desired 1-*O*-acetyl derivative **9** as an anomeric mixture (α/β 1:2 to 1:14). The minor isomer, 4-*O*-acetate **10**, was found to undergo spontaneous elimination of acetic acid to yield exocyclic olefin **11** over a period of several weeks at room temperature (Scheme 1).

Next, N-glycosylation of acetate derivative **9** was accomplished by coupling to thymine in the presence of TMS-trifluoromethanesulfonate as the Lewis acid catalyst (Scheme 1). We propose that the α face of the molecule is partially blocked by a benzoxonium ion resulting from an attack of the benzoate ester on the thiacarbenium ion (Figure 1), as has been observed using other 3′-directing groups.[11] This mechanism would be more favored in nonpolar solvents, where a localized cation is highly unstable (Table 1). Accordingly, our use of nonpolar solvents improves the β/α ratio significantly compared to that reported in the literature for similar Lewis acid catalyzed glycosylations[6] and gives a comparable yield of β product to that obtained via a fusion reaction of the corresponding glycosyl bromide with cytidine.[5]

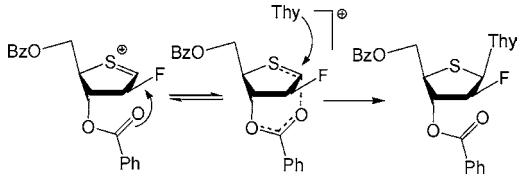


FIGURE 1. Proposed 3′-*O*-benzoate participation in the glycosylation reaction. Increased participation occurs in nonpolar solvents in which the thiacarbenium ion is less stable.

TABLE 1. Anomeric Ratio of Nucleoside Products for Glycosylations with the β-Acetate 9β as Starting Material ($\alpha \leq 10\%$)

solvent	dielectric constant[12]	product α/β ratio
CH_3CN	37.5	3:1
CH_2Cl_2	9.1	1.7:1
$CHCl_3$	4.8	0.9:1
CCl_4	2.2	0.7:1

After the removal of the α nucleoside **12**α by silica gel chromatography, the β nucleoside **12**β was debenzoylated using 2 M methanolic ammonia to give **13** in 87% yield.

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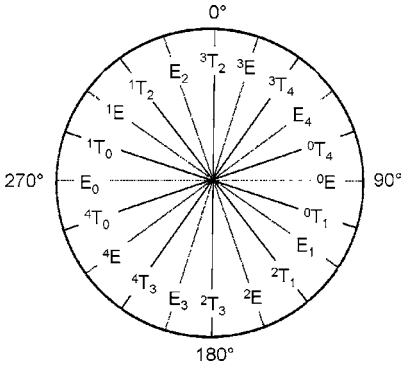

FIGURE 2. Pseudorotational wheel describing the conformations of nucleosides; E = envelope, T = twist. Natural nucleosides have characteristic minima in the north (0°) and south (180°) regions, and interconversion occurs preferentially via the east (90°) pseudorotamer.

TABLE 2. Vicinal ^{1}H−^{1}H and ^{1}H−^{19}F Coupling Constants[a] in 4′S-FMAU (13) and FMAU (14) Nucleosides in D_2O

	4′S-FMAU (**13**)	FMAU (**14**)
H1′−H2′	6.0	4.0
H1′−F2′	7.9	16.9
H2′−H3′	7.1	2.9
F2′−H3′	12.1	19.6
H3′−H4′	7.0	5.0

[a] In Hz.

Conformational Analysis. The conformational parameters of a nucleoside or another furanoside can be described using two parameters, namely, the phase angle *P* and the degree of maximum puckering ϕ_{max}.[13] The value of *P* takes on an intuitive meaning when it is represented on a pseudorotational wheel, as shown in Figure 2.[13]

The vicinal proton−proton and proton−fluorine coupling constants of the fully deprotected nucleoside **13** were examined and compared with those of its 4′-oxygen congener **14** (Table 2). The Karplus equation predicts that a northern conformer of an arabino sugar will have large values of $^3J_{H2'-H3'}$ and $^3J_{H3'-H4'}$, whereas a southern conformer will have large values of $^3J_{H1'-F2'}$ because the nuclei are nearly antiperiplanar in all of these cases. Taken together, the changes in these ^3J values showed that a northern conformer was preponderant for the 4′-thionucleoside.

A large decrease of 7.5 Hz was observed in $^3J_{F2'-H3'}$ upon changing the ring heteroatom from oxygen to sulfur. One obvious explanation for the large $^3J_{F2'-H3'}$ in the 4′-oxo species would be a contribution from an eastern conformer, in which F-2′ and H-3′ are eclipsed. Indeed, this conformation has been shown to exist in 2′F-ANA (4′-oxo) oligomers.[14] This putative eastern conformation would be less significant for the 4′-thio species, according to the reduced value of $^3J_{F2'-H3'}$.

Although this qualitative examination of vicinal ^{1}H−^{1}H and ^{1}H−^{19}F coupling constants is helpful to a certain extent, the conclusions are approximate because of the rapid interconversion of nucleoside conformers at room temperature. We turned, therefore, to the use of the PSEUROT 6.3 program,[15] which is able to account for a two-state equilibrium and provide the pseudorotational parameters for two interconverting conformers. Although some conformational work has been done on nucleoside **14** previously,[16] detailed, empirically derived data was not available, and we therefore undertook a PSEUROT study of both nucleosides **13** and **14**.

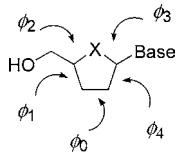

FIGURE 3. Definitions of internal torsion angles in a nucleoside.

TABLE 3. A_j and B_j Parameters for 13 and 14

	4′S-FMAU (**13**)		FMAU (**14**)	
	A_j	B_j[a]	A_j	B_j[a]
$H_{1'}-H_{2'}$	1.098	2.24	1.041	1.14
$H_{1'}-F_{2'}$	1.081	123.24	1.029	122.28
$H_{2'}-H_{3'}$	1.072	119.96	1.150	122.27
$F_{2'}-H_{3'}$	1.076	0.54	1.177	1.77
$H_{3'}-H_{4'}$	1.043	−125.80	1.057	−127.20

[a] In degrees.

Several sets of parameters are necessary for the PSEUROT calculations. Valence angles are not perfectly tetrahedral, and an equation is needed to relate the external torsion angles (therefore, the vicinal coupling constants) to the internal torsion angles (therefore, the pseudorotational parameters *P* and ϕ_{max}). These two sets of angles are related as follows

$$\phi_j^{ext} = A_j\phi_j + B_j$$

for $j = 0, ..., 4$. The definitions of the internal torsion angles are shown in Figure 3. Because these parameters were unknown for 2′-fluoroarabino or 2′-fluoro-4′-thioarabino configurations, we obtained them from DFT calculations[17] (Table 3).

A second set of parameters helps compensate for the nonequilateral nature of the rings. These parameters, α_j and ϵ_j, named after Ernesto Díez, are used to modify the classical pseudorotation equations.[18] Thus, in place of the standard pseudorotation equation

$$\phi_j = \phi_{max}\cos(P + 144°(j))$$

the equation is extended to yield

$$\phi_j = \alpha_j\phi_{max}\cos(P + \epsilon_j + 144°(j)).$$

Including the α_j and ϵ_j parameters in calculations involving 4′-thionucleosides is particularly important because of their greater

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TABLE 4. Diez Parameters α_j and ϵ_j for 13 and 14

	4′S-FMAU **(13)**		FMAU **(14)**	
	α_j	ϵ_j[a]	α_j	ϵ_j[a]
ϕ_1	1.030	−3.615	0.998	1.621
ϕ_2	0.955	−0.355	1.012	0.252
ϕ_3	0.952	0.435	1.016	−0.223
ϕ_4	1.032	3.478	0.995	−1.415
ϕ_0	1.035	−0.057	0.981	−0.229

[a] In degrees.

TABLE 5. Final Results from PSEUROT Calculations (Including $^1H-^{19}F$ Coupling Constants) for 4′S-FMAU (13) and FMAU (14)

nucleoside	P_I (ϕ_{maxI})[a]	P_{II} (ϕ_{maxII})[a]	ratio	rms error of the fit (Hz)
13	−4 (44)	199 (43)	77:23	0.000
14[b]	−6 (36)	126 (36)	31:69	0.595
14[c]	−35 (39)	116 (53)	37:63	0.000

[a] In degrees. [b] With ϕ_{max} of both conformers constrained at 36. [c] With no constraints on the minimization.

deviation from equilateral geometry.[19] These parameters were therefore obtained for both systems studied by least-squares minimization using the DFT-calculated structures mentioned above and the program FOURDIEZ[20] (Table 4).

A generalized Karplus equation has been developed for $^1H-^{19}F$ couplings and proved to be useful for this work.[21] However, because the $^1H-^{19}F$ coupling constant is not as well characterized as the $^1H-^1H$ coupling constant, our initial PSEUROT calculations were carried out using only the three $^1H-^1H$ coupling values. To identify all possible solutions, 2400 consecutive calculations were carried out with different initial values of the five pseudorotational parameters, optimizing three of them at a time. The results were sorted by their rms error, and the best several hundred solutions were examined carefully. Multiple possible solutions emerged (Supporting Information, Table S1).

To differentiate between these possible solutions and to refine the structures, the $^1H-^{19}F$ coupling information was included. Each of the possible regions from the initial calculations was taken in turn as the starting point for the calculations. Inclusion of the fluorine couplings led to one set of pseudorotational parameters for 4′-thionucleoside **13** being easily identified (Table 5). For 4′-oxo nucleoside **14**, the solution of best fit corresponded to a very unlikely arrangement, with the two conformers showing drastically different ϕ_{max} values and the second conformer being too highly puckered for an oxacyclic nucleoside.[22] Therefore, the calculations were also carried out constraining the ϕ_{max} of both conformers to 36°, a likely value according to the computed structures. The phase angles and mole fractions obtained from these two sets of calculations were similar; both results are listed in Table 5.

Regardless of which of the two solutions best describes nucleoside **14**, it is clear that, as predicted by the qualitative examination of coupling constants, a northern pseudorotamer is preponderant for **13**, whereas **14** is dominated by a conformer remarkably close to the southeast (Figure 2).

The reasons for this dramatic conformational change are complex. The steric effects between the thymine base and the sugar ring would be reduced for a 4′-thionucleoside with its longer C−S bonds, thus favoring a north conformation in which the base is pseudoaxial. O−C−C−X gauche effects are typically of greater magnitude than S−C−C−X gauche effects,[23] and accordingly, we would expect greater $\sigma_{C3'H3'} \rightarrow \sigma^*_{C4'O4'}$ and $\sigma_{C2'H2'} \rightarrow \sigma^*_{C1'O4'}$ interactions relative to the $\sigma_{C3'H3'} \rightarrow \sigma^*_{C4'S4'}$ and $\sigma_{C2'H2'} \rightarrow \sigma^*_{C1'S4'}$ overlap. One predicts, therefore, that the gauche effects would also provide a strong driving force for the south (or east) conformation in the case of the oxygen congener. However, a greater anomeric effect in the case of the oxygen congener[23,24] would favor the north conformation. It follows then that the observed conformational preferences are dominated by steric and gauche effects.

It is of interest to note that whereas 4′S-FMAU (**13**) adopts predominantly the north conformation, the 2′-deoxynucleoside, that is, 4′-thiothymidine (4′S-dT), adopts a south conformation in the solid state and a predominantly south conformation in solution.[19] However, in the latter case, evidence was presented for the representation of the north conformation in the conformational ensemble.[19] The shift from a predominantly south conformation in 4′S-dT to the north conformation in **13** must be caused by the greater F2′ steric effect in the south conformation that outweighs the stabilization gained by the F2′−S4′ and O3′−S4′ gauche effects in 4′S-FMAU.

Evidence for an accentuated steric effect in arabinofuranosyl nucleosides in the south conformation may be inferred by the significant population of the north conformation in 4′-thioarabinoadenosine (4′S-araA).[25] We propose that this unfavorable interaction is derived from syn−axial interactions between the CH_2OH moiety at C-4′ and the substituent at C-2′. We note that base-modified 2′F-arabinonucleosides with north as well as southeast conformations have been reported by Seela and co-workers.[26]

Conclusions

Conformational analysis of 2′-deoxy-2′-fluoro-5-methyl-4′-thioarabinouridine (4′S-FMAU) showed that it adopted north (−6°) and south (199°) conformations with a 77% preference for the north. This is in sharp contrast to the southeast (∼120°) conformer that dominates the conformational equilibrium of its 4′-oxygen congener (∼65%). Arguments are presented to suggest that the replacement of oxygen by the cognate sulfur atom at the 4′ position leads to a decrease in the magnitudes of C5′-base steric effects and various gauche effects and a corresponding shift to a north conformation.

Experimental Section

Parametrization of PSEUROT for 2′-Fluoroarabino Configurations. A_j and B_j parameters for these two systems were obtained using a method similar to that of Houseknecht et al.[27] All

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DFT calculations were carried out on a PC using the Gaussian 03W program.[28] All optimizations were carried out in the gas phase.

For FMAU nucleoside **14**, a series of 32 envelope structures was optimized at the B3LYP/6-31G** level. In each case, one of the torsion angles $\phi_0-\phi_4$ was constrained to zero to span the full range of *P* accessible to nucleosides. The starting value of the glycosidic torsion angle X (C2−N1−C1′−O4′) was also constrained to various values, covering the full range of conformational space.

For 4′S-FMAU nucleoside **13**, a series of 12 envelopes spanning the pseudorotational space was minimized at the B3LYP/3-21G** level. Starting structures were set to a ϕ_{max} value of 35°. The value of the glycosidic torsion angle X (C2−N1−C1′−S4′) was initially set to 220° in all cases because this value is in the middle of the anti range, where the minimum conformation of thymidine nucleosides is expected, especially given the presence of the 2′-fluoro substituent. The O5′ was set anti to C3′, and OH3′ and OH5′ were set anti to C4′. Optimizations were carried out constraining only one torsion angle $\phi_0-\phi_4$ to zero.

The internal and external torsion angles were graphed (Supporting Information, Figures S1−10) with linear plots having a slope of A_j and a *y* intercept of B_j. The resulting A_j and B_j parameters are given in Table 3.

PSEUROT Calculations. Proton spectra were zero filled to 128 K and resolution-enhanced with Gaussian or sinebell functions. The vicinal coupling constants were then extracted directly. For the FANA species, the spectra were measured at various temperatures, but only minute changes in the coupling constants were observed up to 40°C.

For the PSEUROT input files, all parameters were calculated as described above except for the empirical group electronegativities, which were obtained from the literature.[29] The initial studies of 2400 different starting values were accomplished using PSEUROT's MANY function that allows the automation of this repetitive task.

In all ^{19}F calculations, the $^{1}H-^{19}F$ coupling was given a weighting of 0.2 to compensate for the inherently larger value of its coupling constant and to give it slightly less weight because the parametrization of the corresponding generalized Karplus equation is less reliable. The FCC and HCC angles along the path of the coupling were chosen according to the method of Mikhailopulo et al.[30]

The initial calculations were executed both with and without the Diez extension of the pseudorotation equations. For both molecules, the results obtained with and without the Diez parameters were similar; however, the rms error tended to be slightly lower for calculations involving **13** and **14**, where the Diez parameters were included and excluded, respectively. Therefore, for the final calculations, the Diez extension was used only for 4′S-FMAU nucleoside **13**.

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Supporting Information Available: Experimental synthetic methods, characterization of compounds **10** and **11**, torsion-angle graphs used to obtain A_j and B_j, and solutions from the first set of PSEUROT calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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